THE INFLUENCE OF ILLUMINATING GAS AND ITS CONSTITUENTS ON CERTAIN BACTERIA AND FUNGI

BY

C. A. LUDWIG

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy, in the University of Michigan

Reprinted from the American Journal of Botany, 5: 1–31. January, 1918

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THE INFLUENCE OF ILLUMINATING GAS AND ITS CONSTITUENTS ON CERTAIN BACTERIA AND FUNGI*

C. A. LUDWIG

INTRODUCTION

It has been known ever since the observations of Girardin (6) in 1854 that certain phanerogams are susceptible to injury by the presence of illuminating gas in the soil or air. Since then considerable work has been done in both Europe and America on the question and on the allied one of the toxicity of smoke. One of the outstanding results of the later work has been a determination of the large rôle played by ethylene in the results observed and of the exceedingly small amount of ethylene which is necessary to bring about the reactions. It has been found, for instance, that the almost infinitesimally small amount of one part of ethylene in 2,000,000 parts of air causes closing of carnation flowers in 12 hours (1) and that the even smaller ratio of one part in 10,000,000 parts causes nastic curvatures in castor-bean seedlings (1).

With the bacteria and fungi, however, there have not been reported thus far any cases where such remarkable sensitiveness to the chemically more inert organic gases has been exhibited. In fact, very little has been done with these gases in this field; and, in most cases reported, gases were used in the pure condition, very few or no attempts having been made to determine the lower limit of toxicity. It became, therefore, a matter of considerable scientific interest and some practical importance as affecting laboratory practice to determine as nearly as possible the lower limit of toxicity of illuminating

* Publication No. 167 from the Botanical Department of the University of Michigan.

gas and its separate constituents toward several of these organisms. The chief aim of this study was to make such determinations.

HISTORICAL

A number of investigations have been carried out to determine the reactions of phanerogams to low concentrations of different gases, and a smaller number to determine the more fundamental matter of the effect on the life processes. They have often been concerned in the first instance with smoke injury; and the results in general have tended to show, as would be expected, that mineral acid oxides or the oxides of toxic elements, as, for instance, arsenic, are decidedly toxic under conditions of much dilution in the air. They have also shown, as mentioned earlier in this paper, that certain plants show a remarkable sensitiveness to certain gases which are ordinarily considered to be quite inert. This work will not be reviewed further here, as it has only an indirect bearing on the problem investigated. A bibliography may be found in papers by Crocker and Knight in the *Botanical Gazette* (1, 2).

When we come to the lower plants, however, we find that comparatively little has been done along this line. Quite early in the history of bacteriology the question of the oxygen relation was worked out and methods were elaborated for its study with relation to any particular organism. This latter work involved a study of hydrogen and carbon dioxide as determining their availability for displacing the air in anaerobic culture conditions; but the matter of other gases, especially in less concentration than purity, appears to have seemed of little or no importance. There have been some pieces of work done, however, which have a suggestive or direct bearing on the problem and are therefore worthy of mention here.

Perhaps the earliest published paper of the kind was by Tassinari (15). In this case, the effect of tobacco smoke on several bacteria, including both pathogenic and non-pathogenic species, was investigated. The exposure to the smoke was made by means of a clever bit of apparatus in which a drop of the culture was held on a fragment of linen and the smoke drawn past it. The strip was then dropped into sterile medium and the time required for development noted. The check cultures developed uniformly in twelve to twenty-four hours. The smoked cultures with only one exception were delayed from twenty-four to one hundred hours in development or had failed to develop at all at the end of eight to twelve days.

Frankland (4, 5) investigated the effect of hydrogen, carbon dioxide, carbon monoxide, nitrous oxide, nitric oxide, sulphuretted hydrogen, and sulphurous anhydride on *Bacillus pyocyaneus* and the spirilla of Koch and Finkler. In carbon monoxide the spirilla produced colonies sparsely while *B. pyocyaneus* produced none until later exposed to the air. Nitrous oxide hindered the production of colonies, but did not prevent it, while nitric oxide, sulphuretted hydrogen, and sulphurous anhydride each prevented the development of colonies not only while the medium was exposed to the gas but also after its return to the air.

Krause (9) observed that *B. pyocyaneus* would grow in an atmosphere of illuminating gas or hydrogen sulphide but would not produce pigment under those conditions. When later exposed to atmospheric air the cultures produced the usual pigment.

Smith (14, p. 58) has made the statement that the small amount of carbon monoxide present where the oxygen has been removed by the potash-pyrogallol method of conducting anaerobic cultures is harmless to many bacteria, but that he has reason to think that it is injurious to others, even if it does not entirely inhibit growth. The grounds for his suspicion were not given.

Molisch (11) studied the effect of tobacco smoke on certain phanerogams and micro-organisms. He found that the movements of *Chromatium vinosum* (Ehrenb.) Winogradsky, *Beggiatoa* sp., and *Spirillum* sp. were stopped by the smoke. The growth of *Phycomyces nitens* was slowed down. His work showed that nicotin will not cause reactions in the phanerogamic plants used, *Vicia sativa*, *Pisum sativum* and *Cucurbita Pepo*, similar to those caused by the smoke, but that pyridin and carbon monoxide will each do it. This result is reinforced by the added observation that the smoke from burning paper, wood or straw will induce the same reactions.

Münz (12) has recently succeeded in isolating from garden soil, ditch water, river ooze and leaf fragments of various water plants certain bacteria which are capable of utilizing methane as a source of carbon and of energy. The writer regrets that he has not had the opportunity of reading Münz's paper. The note given above was made from an abstract in the *Zeitschrift für Botanik*. The original paper is a dissertation at Halle, and, owing to the war, was not available for examination.

The published work with fungi and algae is even less abundant than with bacteria. A few pieces of work are worth mention, however.

Molisch has shown (11), as was mentioned above, that the growth of *Phycomyces nitens* is slowed down by smoke; and Thom (16) has reported that in an atmosphere of carbon dioxide no one of the species of Penicillium with which he worked showed growth within a week but that development set in after the tubes were restored to the air.

Richards and MacDougal (13), working with Nitella, found that this plant could live in carbon monoxide of 80 percent concentration but it was somewhat paler than the check in air.

Working with certain other algae, Woycicki (17, 18) has shown that illuminating gas will induce certain remarkable alterations both in the shape of the cell and in its internal structure. With species of Spirogyra, Cladophora and Mougeotia he found that in many cases curious outgrowths of the cells were produced which often resembled holdfasts, while the contents of the cells became more or less disorganized, according to the strength of the gas. The cells, in fact, were often killed; and the filaments usually became broken up into small pieces or even into the individual cells. *Cladophora fracta* var. *horrida* showed a much smaller degree of sensitiveness than Spirogyra. It was found also that the laboratory air often contains enough gas to induce alterations in the algae and that carbon monoxide and acetylene are capable of calling forth the changes.

Langdon (9, 10) has recently made the somewhat remarkable discovery that free carbon monoxide occurs in the floats of a Pacific marine alga, *Nereocystis luetkeana*, sometimes to the extent of 12 percent of the enclosed gases. The range was found to extend down to 1 percent, while the average was about 4 percent. This is interesting in view of the generally accepted belief in the poisonous nature of carbon monoxide to plants, since it shows that at least some plants capable of conducting photosynthesis contain tissues which are tolerant of quite large amounts of the gas.

INVESTIGATION

Organisms Used and General Methods

The organisms used in the study here reported consisted of bacteria and fungi. Of these, a number, *Bacillus subtilis* Cohn, *B. Kieliensis* (Lehm. and Neum.) Mig. ("ruber of Kiel"), *B. pyocyaneus* Gessard, *B. rubidus* Eisenberg and *Sarcina lutea* Schröter, were obtained from the department of bacteriology of the University of Michigan. A

number of others, *Bacillus carotovorus* Jones, *B. melonis* Giddings, *B. campestris* Pammel, *B. mycoides* Flügge, *B. solanisaprus* Harrison, *Pseudomonas radicicola* (Bey.) Moore, *Bacterium stewarti* Erw. Smith and *B. tumefaciens* Erw. Smith, were secured from the American Museum of Natural History through the botanical department of the University of Michigan. The following fungi were used: *Oidium lactis* Fresenius, obtained from the department of bacteriology, a strain of *Penicillium stoloniferum* Thom, isolated from moldy bread and determined by Miss Margaret B. Church, of the U. S. Department of Agriculture, a previously undescribed yeast,[1] isolated from the air at the University of Michigan, *Penicillium pinophilum* Hedgcock, *P. camemberti* Thom, *P. roqueforti* Thom and *P. expansum* Link, the last four of which were obtained from Miss Margaret B. Church through the courtesy of Dr. Charles Thom, and the four species, *Fusarium radicicola* Wollenw., *Gleosporium cingulata* Atkinson, *Endothia parasitica* (Murr.) P. J. & H. W. And. and *E. fluens* (Sow.) S. & S., which were received from Dr. Lon A. Hawkins.

The cultures were carried on ordinary 1 percent glucose, 1 percent peptone, 0.3 percent beef extract agar, with 0.5 percent sodium chloride and 1.5 percent agar, except that some of the experiments with *B. rubidus* were carried out on autoclaved potato slants. The color which *B. rubidus* develops on this substratum made the medium of

[1] I take this opportunity of thanking Dr. H. W. Anderson for help with this species. He has made a taxonomic study of it and has kindly furnished the following diagnosis:

Cryptococcus Ludwigi H. W. Anderson sp. nov.

Morphology.—Cells round or oval, becoming elliptical in old cultures. Cytoplasm very coarsely granular. A single large granule usually evident. Buds arising from any point but usually from shoulders in elliptical cells. Size 3.5 × 4.5 μ.

Cultural characters.—On dextrose agar the streak is filiform, at first light pink, slimy, smooth, later becoming dry and very decidedly wrinkled and heaped. The dry, wrinkled type of growth is peculiar to this species of pink yeasts. On carrot and other solid media the streak has the same type of growth. In gelatin stab the line of puncture is filiform with no liquefaction.

Biochemical properties.—There is no fermentation of dextrose, lactose, galactose, sucrose, levulose nor raffinose. Litmus milk is rendered more alkaline.

From culture No. 51. Type specimen No. 51. Type slide No. 51. Cultures have been sent to several laboratories; and type slide, culture and dried material have been deposited in the herbarium of the University of Illinois. The organism was isolated from the air at the botanical laboratories of the University of Michigan, Ann Arbor, Michigan.

some value. The reaction of the agar varied with the different lots made up from nearly zero to slightly over + 1 on Fuller's scale, but was usually about + 0.8.

The exposures of the bacteria and some of the fungi to the gases were made in cotton-plugged test tubes confined in airtight chambers. This method has the disadvantage that it is practically impossible to get quantitative data by its means, such as could be obtained by using Petri plates and counting colonies, but the development can be followed better from day to day in tubes than in plates within a larger vessel. In most of the work it was a great advantage to be able to observe the cultures easily without removing them from the gas. The airtight chambers used consisted of four Novy jars and a number of bell jars with tubulature at the top which were fitted with perforated rubber stoppers holding tubes for the introduction of gas. Each bell jar was placed in a base composed of a heavy crystallizing dish with a layer of plaster of Paris about 2 cm. thick, impregnated with paraffin, in the bottom. The plaster of Paris was prevented from breaking the dish in setting by putting paraffined corrugated paper next to the wall of the dish in order to take up the expansion. The chambers were sealed by running melted paraffin between the case of the bell jar and the wall of the dish.

During the earlier part of the work, the gases were introduced from a Hempel gas burette by means of the pressure of a few centimeters of water. However, in most of the experiments the gas was allowed to enter directly and its amount was regulated by means of a mercury manometer. The reservoir for the mercury in this case was a wide-mouthed bottle closed by a two-hole rubber stopper through one hole of which the tube containing the mercury column passed. By means of a second glass tube through the other perforation in the stopper the apparatus was easily attached to any chamber in which the gas pressure was to be measured. When a certain amount of gas was to be introduced into a given chamber, the chamber and the manometer were connected at the same time to an aspirator and exhaustion was carried out, usually to about 15 cm. of mercury. The apparatus was then connected to the gas container and gas was allowed to enter until the pressure had risen the calculated amount on the scale, the calculation being on the basis that the amount of gas in a given volume varies directly as the pressure. The apparatus was then allowed to finish filling with air, after which it was closed

and set aside. The reason for introducing the gas while the pressure was low was to secure the vacuum as an aid to distribution in getting the gas through the plugs into the tubes in contact with the cultures. Some accompanying experiments with tobacco smoke and methyl iodide vapor, in which diffusion alone was relied on to get the gases into the tubes, gave results in the resulting cultures which showed that the gases did get into the tubes, at least in small amounts, very promptly. It seems not unreasonable, therefore, to think that the composition of the gas within the tubes approached pretty closely that in the rest of the chamber, although it was impossible, of course, to get absolute data on the point. The concentrations mentioned in all cases are to be considered not as exact values but merely close approximations. When pure gas of some kind was desired in contact with the cultures, one of two or three different plans was employed. In the case of illuminating gas, it was either allowed to pass through the vessel continuously during the experiment or it was passed through rapidly for one to two hours and then stopped. In the latter case it was usually renewed daily during the experiment. For other gases, which had to be manufactured for the purpose, the test tubes containing the cultures were fitted with perforated rubber stoppers through which small glass tubes passed. These test tubes were arranged in a chain and the gas was passed directly through them. The stoppers were sealed to the glass with which they were in contact by means of sealing wax, and the rubber connections between tubes were carefully wired and paraffined.

All results here reported, unless otherwise stated, were from at least two trials; and many of them were checked several times.

EXPERIMENTAL

1. Illuminating Gas

Source and Composition of the Gas

A large part of the work consisted of tests with illuminating gas. Such tests have the disadvantage, of course, that the gas is a mixture, and not a perfectly constant mixture at that; but its ready availability and the fact that it is the substance which usually contaminates laboratory air made it seem worth while to use it. The gas used was taken from the gas taps in the laboratories and was the same as

that used throughout the city of Ann Arbor. During the first part of the experiments (winter of 1915–'16) it was pure coal gas;[2] later (winter of 1916–'17 to Feb. 1), it was a mixture of coal and water gas; and at the last (after Feb. 1, 1917) it consisted once more of coal gas only, except for the 5-day period, February 12–16, during which time a small amount of water gas was mixed in. The gas before and after February 1, 1917, analyzed approximately as follows:

	Before Feb. 1	After Feb. 1
CO_2	1– 2%	0.9–2.0%
C_nH_{2n}	4– 5%	3.5–4.5%
O_2	1– 2%	0.8–1.5%
CO	11–14%	6.0–7.8%
CH_4	25–30%	30– 35%
H_2	40–50%	35– 45%
N_2	about 10%	8– 11%

The figures given here are not the result of specific analyses made for the purpose of this study, but are the result of the examination of a large number of student analyses. However, as the gas was used at various times over an interval of a year and a half or more, it seems that a more exact analysis would be little or no more valuable for interpreting the results.

Ordinarily the gas was not washed. It was the original intention to do so, but this could not conveniently be done because the pressure in the pipes was too small to drive the gas through wash bottles. Moreover a few preliminary experiments with the gas showed that it did not exhibit extraordinary toxic properties toward the organisms used. It was therefore decided that as long as no very great toxic properties were shown it was not necessary to remove traces of H_2S, NH_3, or other inorganic gases which presumably might be present and exert harmful influences.

Effect on the Different Organisms

In giving the results of the tests with illuminating gas, and with the other gases as well, a brief summary will be given for each species used, instead of giving a chronological account of the experiments or of giving single experiments in detail.

[2] I am indebted to Prof. W. L. Badger, of the department of chemical engineering of the University of Michigan, and to Mr. Chas. R. Henderson, chemist to the Washtenaw Gas Co., for the analyses and data given here concerning the illuminating gas used in these tests.

Bacillus subtilis.—This organism was cultivated in the following approximate concentrations of illuminating gas: 0.5 percent, 5 percent, 10 percent, 25 percent, 50 percent, 75 percent, 85 percent, and 100 percent. In concentrations up to and including 25 percent, the colony development, both as to abundance and as to character, was practically identical with the development in air. This normal growth, as is well known, consists of a white, often wrinkled layer on the surface of the agar. The development in 50 percent gas and above, however, was quite different in character. The chief difference, and perhaps the only one of importance, was the much smaller mass of the colony produced. The colony was always very thin, so that it never had the opaque character of normal ones. The development was confined to the inoculated area and did not extend over the surface of the agar as was the case when development was normal. Occasionally only pin-point colonies were developed, or perhaps nothing at all until after return to the atmosphere. Complete sterilization practically never took place with an exposure not to exceed ten days; but it sometimes took a week or more for development to become evident after return to the atmosphere. In those cases where some development occurred in the gas it did not proceed further when returned to the air, but usually after the lapse of a variable period of time an area of normal development began at some point and grew over the slant. Inoculations made from these colonies grown in gas produced in the air a colony development differing very slightly or not at all from the normal in appearance.

Two series of experiments were run to test the ability of the organism to grow continuously in different percentages of illuminating gas. In these tests the inoculations after the first were made from cultures in the same concentration to which they were to be exposed unless no development had taken place in that concentration. In that case the inoculation was made from the highest concentration at which growth had occurred. In the course of this work, the organism was carried through 5 transfers in each of 5 percent, 10 percent, and 25 percent gas, 3 transfers in 50 percent, 5 transfers in 75 percent and 85 percent, and 9 transfers in pure gas. It is quite evident, therefore, not only that the organism can grow in the gas, but that it can continue so to grow for an indefinite time.

The growing of the organism in the pure gas seems to have caused little or no change in it, except in the colony character due to the

slowing down of growth to a very low point. This is evidenced by the fact, mentioned above, that a culture from a line previously carried in the air developed as well in the gas as another culture from a line previously carried in gas. It is also evidenced by the fact that in the first transfer into the air after a period of several transfers in gas the colony growth was normal in appearance in all respects. The examination of stained microscopic mounts supported the foregoing evidence. Bacteria from air cultures one day old and 11 days old and from a gas culture 11 days old were stained. In size, shape and especially in the absence of spores the bacteria of the gas culture (11 days old) resembled those of the one-day air culture more than they did those of the 11-day culture. There were only a few spores in the one-day culture, none in the 11-day gas culture, but most of the structures in the 11-day air culture were spores.

Bacillus pyocyaneus.—The culture of *B. pyocyaneus* used in this study developed the color only rarely. The color has therefore not been used as a character on which to base comparisons, although it has been so used in the past. The typical colony growth was rather dirty white, semi-translucent in character; and it was often difficult, because of the indefinite tint of the colony, to detect differences in the development of the growths under comparison. This organism was first grown in 5 percent, 50 percent, and 85 percent gas. The development was quite normal in 5 percent gas, but proceeded more slowly in the higher concentrations, so that whereas it took about three days for a culture in air to reach its maximum, it took two to three days longer for the 85 percent gas culture to reach the same stage. Attempts at this time to grow the species in pure illuminating gas met with three clear-cut failures and one apparent success, which, however, was possibly due to failure to displace all of the air in the container. In one of the cases of failure the organism developed (after a 4-day exposure) when returned to the air, but in the other two (after exposures of 6 days and 3 days) it did not develop within periods of 16 days and 27 days respectively. In later experiments, as will be shown presently, the attempt to cultivate the organism in pure gas resulted successfully. There was little or no alteration in the colony character in the gases, provided a colony was produced.

It was found also that the organism can apparently be carried indefinitely in most of the gas concentrations and perhaps even in pure gas. It was carried through 5 transfers in 5 percent, 10 percent,

and 25 percent gas, 3 transfers in 50 percent, 5 transfers in 85 percent, and 9 transfers in pure gas. In the unsuccessful trials with pure gas in the later work the organism always developed on the slant after being exposed to the air, although sometimes appearing in separate colonies instead of a streak, as if most of the inoculating bacteria had been killed. When success was attained in cultivating the organism in pure gas it then seemed likely that the bacillus had in some way developed the ability to grow under those conditions which were at first inhibitory; but this hypothesis was found to be untenable when trial was made by inoculating from a line which had been cultivated in the air only, for these cultures developed just as well as the ones which had been carried in gas for several transfers. These apparently contradictory results seemed quite unexplainable except on the assumption of a change in the composition of the gas, and it was thought at the time that no significant change had taken place. Later, knowledge was obtained of the variation in the gas concentration which has already been noted. Upon comparison it was found that the successful cultures of the organism began about Feb. 1, 1917, at the time the change was made to pure coal gas instead of the mixture of coal and water gas. The significant change in the composition of the gas would seem to have been the drop from about 12 or 13 percent to 7 or 8 percent of carbon monoxide. It should be remarked, however, as will be shown later, that neither of these concentrations of CO is of much significance if the rest of the mixture be atmospheric air. In fact, the tolerance of the organism to CO-air mixtures is so great that one would not expect a difference of only 5 or 6 percent in the concentration to exert any marked effect. It is also worthy of note that the first failure to grow in the gas occurred during the first period when the gas consisted of pure coal gas and had the lower carbon monoxide content. The results, therefore, are even yet unexplainable with the data at hand.

Bacillus Kieliensis.—This organism grows vigorously, reaching a maximum in 3 to 4 days, and has a very brilliant red color with a strong greenish metallic or coppery sheen on the surface. After the colony has reached its maximum the sheen gradually disappears, the colony becomes brickish red, and the pigment often diffuses more or less into the medium. This color responds readily to cultural conditions and so furnishes a sensitive index for detecting disturbances of the life processes. Its alterations are so numerous and complicated,

however, that no attempt will be made to describe them fully or to mention the many variations observed. In gas concentrations of 10 percent and less the development was normal. In 25 percent it was sometimes normal but more often slightly retarded and the color rendered less brilliant. In 50 percent gas the metallic sheen was usually nearly lacking, the color considerably lighter, and the rate of growth considerably less, so that it took 2 or 3 days longer for it to reach its maximum than it took in air. In still higher concentrations these changes were progressively more noticeable until pure gas was reached, in which surroundings development was very slight and the colony colorless or whitish, with only occasionally a trace of pink. In the gas-air mixtures the color was usually variable, ranging from deep red to light pink or whitish and usually with purple shades; and a number of these tints usually occurred in the same streak. The weak colonies which developed in gas of a high concentration grew vigorously and developed pigment when returned to the air, but the pigment never reached the depth of color shown by a colony grown in air from the start.

The organism was carried continuously through 5 transfers in 5 percent, 10 percent, 25 percent, and 85 percent gas, 3 transfers in 50 percent, 11 transfers in 75 percent, and 10 transfers in pure gas. Cultures in the air inoculated from cultures carried for several transfers in pure gas grew rapidly and developed abundant but not normal pigment, although the pigment production returned to normal after a few transfers in the air. Where the cultures had been carried in gas for only two transfers the color was normal at the first recultivation in air.

The examination of stained preparations failed to show any striking differences between the treated and untreated bacteria. In air culture the organism tends to show shorter, smaller, more coccus-like rods as the culture grows old. In gas the juvenile shape seems to be maintained for a longer period of time, probably owing to the slowing down of the development.

Bacillus rubidus.—Part of the cultures of *B. rubidus* in illuminating gas were made on autoclaved potato plugs. On this medium a clear orange color is produced which makes development easy to detect. The organism grew well in 0.5 percent and 5 percent illuminating gas; but did not grow in a strength of approximately 85 percent, except possibly in one trial, although it developed promptly when

restored to the air. In pure gas no development occurred during the exposure. In one case no development followed a 3-day exposure within 18 days after removal, and in another a 6-day exposure was followed by no development within 22 days, while in a third a very slight development began 5 days after the close of an 8-day exposure.

Sarcina lutea.—This proved to be one of the more susceptible organisms studied. It was, however, grown in 5 percent, 10 percent, 25 percent, 50 percent, 75 percent, and 85 percent of illuminating gas. There was ordinarily no checking of the development in the 5 percent concentration, but the growth gradually became less in all higher concentrations used. In all tests the air cultures reached their full development first, followed in succession by the others; but only rarely in 50 percent and above did the maximum in gas equal the maximum in air. The organism was carried continuously through 5 transfers in 5 percent, 10 percent, and 25 percent gas, 3 transfers in 50 percent, and 10 transfers in 75 percent. It was found impossible to secure unmistakable development in pure gas although there were a few cases of possible very slight development.

Oidium lactis.—*O. lactis* showed about average resistance to the effects of the gas. It was grown in all concentrations of illuminating gas employed, but its behavior in the stronger concentrations was rather erratic. Usually the growth under such conditions was very slight; and sometimes it started from only a few isolated points along the streak, as if the treatment had partially sterilized the slant; but at other times it would approach the maximum reached by a culture in air if left long enough. It was grown continuously for 5 transfers in 5 percent, 10 percent, and 25 percent gas, 3 transfers in 50 percent, 11 transfers in 75 percent, and 5 transfers in 85 percent and 100 percent. At times the character of the colony development in large percentages of gas differed from that in low percentages or in air, but here again the reaction was not uniform. In some tests the mycelium was more appressed and water-soaked in appearance in a high gas atmosphere and in others it was more upright and tufted or white velvety in appearance.

Cryptococcus Ludwigi.—This organism did not show any particularly remarkable characteristics in connection with these studies. Normally the colony is deep pink in color and is composed of quite a considerable mass of material. In gases the toxic effects are evidenced by a retarded or incomplete development of the colony and by a

paler color than normal. The organism grew in all the gas-air mixtures used—5 percent, 10 percent, 25 percent, 50 percent, 75 percent, and 85 percent gas—but not in pure gas. The 5 percent and usually the 10 percent concentration did not show any toxic effect, but at 25 percent of illuminating gas the development was always checked. At 75 percent and 85 percent the development was very slow and the colony quite pale, sometimes nearly colorless. Normally an exposure in pure gas was followed by development within a week when returned to the air, but in a few cases an exposure of a week or less seemed to sterilize the material, since no development had taken place in 13–18 days. The organism was carried through 5 transfers in 5 percent, 10 percent, 25 percent, and 85 percent gas, 3 transfers in 50 percent, and 11 transfers in 75 percent.

Penicillium stoloniferum.—This species grows rather rapidly on the medium used and soon becomes green with the large number of conidia produced. This color soon changes to some shade of brown, and later the colony is often overgrown with hyphae from underneath. The checking effect of unfavorable conditions can often be detected for several days after conidia production by means of the younger appearance of the retarded cultures as compared with the check. Development occurred in 5 percent, 10 percent, 25 percent, 50 percent, 75 percent, and 85 percent gas, but not in pure gas. It was quite normal in character to 50 percent but was much slowed down at that concentration. The checking of growth was observed at 10 percent but not at 5 percent. At concentrations of 75 percent and above development was slow and did not extend very far laterally, while a good many of the spores were apparently killed. As a result a cushion-shaped or roughly hemispherical mass of apparently upright hyphae was produced at each point of inoculation. The entire slant, therefore, often contained these pulvinate colonies, which sometimes reached a diameter of 2 or 3 mm. and became more or less confluent. Conidia were not produced under such circumstances except in one or two instances in which it is doubtful if the percentage of gas had been maintained. Even after the return of these cultures to the air there was only exceptionally any conidia production or other growth, although new cultures inoculated from them and kept in the air developed conidia normally. Cultures prevented from developing by being exposed to pure gas grew and produced conidia as usual in some cases upon being returned to the air. The species was maintained

continuously for 4 transfers in 5 percent, 10 percent, and 25 percent gas, 3 transfers in 50 percent, 10 transfers in 75 percent, and 4 transfers in 85 percent illuminating gas.

Bacterium stewarti.—*B. stewarti* is an aerobic organism which proved to be one of the most susceptible employed. It grew in 5 percent, 10 percent, 25 percent, 50 percent, and 75 percent of illuminating gas; and it was possible to keep it growing continuously in these; but the development in the last concentration mentioned was slow. A distinct checking effect was always shown at 10 percent and often a slight one seemed to be present at 5 percent. In the earlier work no development was secured at 75 percent, but later on growth did occur. The first development occurred at about the time the gas company ceased producing water gas, as was the case with the first development of *Bacillus pyocyaneus* in pure gas. The circumstance was probably due to the reduction of the CO content of the gas. No development was observed in any concentration used above 75 percent; but only rarely did development fail to take place after removal to the air, although it was usually 5–15 days in becoming visible and was also usually slight or very slight in amount.

The following organisms were tested once in each of 25 percent, 50 percent, 75 percent, and 85 percent, and twice in 100 percent illuminating gas.

Bacillus carotovorus.—With this organism there was slight development by the end of a 6-day exposure to pure gas in one test but none in the other at the end of an equal period. In both cases, however, prompt development followed a return to the air. Growth occurred in all the lower concentrations, but it was checked in all; and the retardation was still noticeable in the 25 percent concentration at the end of the 6-day period.

Bacillus melonis.—In one case with *B. melonis* the tube in pure gas showed a very slight development at the end of the period. In the other, however, no growth was visible although it became so soon after the return to the air. Growth took place in all the lower concentrations but it was much retarded in all.

Bacillus campestris.—With *B. campestris* also, development occurred in all the concentrations used except pure gas, although there was distinct retardation in even the 25 percent concentration. In pure gas there was no growth in one trial but a possible very slight development in the other. In both cases growth occurred after

removal from the gas, although it took periods of 6 and 5 days respectively for it to become discernible.

Bacterium tumefaciens.—There was visible development of *B. tumefaciens* in all percentages of gas except pure gas, although a distinct checking effect was observed in the lowest concentration used, 25 percent. No development took place in pure gas, but it occurred after returning the tubes to the air. The colonies in this case became visible in 5–8 days after the removal from the gas.

Bacillus solanisaprus.—There was a distinct and considerable checking of the development of *B. solanisaprus* in all of the concentrations of gas used, and in the greater ones the development was only slight. In the pure gas there was no visible growth, although it did occur following the return of the cultures to the air, in which case it became visible in 1–5 days.

Pseudomonas radicicola.—*Ps. radicicola* proved to be one of the more susceptible species. Development occurred in 25 percent gas, but it was only slight. At 50 percent concentration and above development was absent, although it occurred following the return to the air. The periods of time in which the colonies became visible in the cultures removed from pure gas to the air were 12 days and 5 days respectively.

Bacillus mycoides.—There was at least a very slight development of *B. mycoides* in all of the concentrations of gas used, but the 25 percent strength showed a slight retarding action, since it took 3 days for the colony to cover the slant from a spot inoculated in the center while in the air the slant was covered in 2 days. The development remained very slight in the higher percentages throughout the 6-day duration of the tests. The very slight colony developed in the case of the 50 percent and 85 percent gas, where the inoculation was by streak, was very similar in appearance to that of *B. subtilis* in concentrated gas. After being removed to the air normal development began in from 2 to 5 days and soon covered the slants. It did not originate all along the streaks, however, but at isolated points, so that separate colonies were formed, as if a partial sterilization of the slant had been produced by killing the inoculating bacteria between the points where the colonies arose.

A number of fungi were grown in Petri plates and the effects of illuminating gas noted, chiefly by measuring the diameter of the colonies at 3-day intervals (in some instances the sum of two different

TABLE SHOWING THE EFFECT OF DIFFERENT CONCENTRATIONS OF ILLUMINATING GAS ON THE GROWTH OF SEVERAL FUNGI

Species	Gas Conc.	First Trial				Second Trial				Third Trial			
		3d Day	6th Day	9th Day	12th Day[1]	3d Day	6th Day	9th Day	12th Day[1]	3d Day	6th Day	9th Day	12th Day[1]
Penicillium pinophilum	Air	14	28	43	58	6.5	24	41	56	9.5	20	35	56
	5%	—	—	—	—	7	24	39	53	10	23	35	50
	10%	—	—	—	—	6.5	21	36	51	8.5	20	33	49
	25%	10	19	29	—	4	16	27	42	8	18	30	47
	50%	8	13	22	—	2	11	21	36	4	10	17	30
	75%	4	5.5	7.5	21	2	4.5	7	22	4(?)	6	8.5	26
	100%	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium camemberti	Air	13	25	35	—	11	23	32	43	11	23	35	45
	5%	—	—	—	—	11	24	32	44	12	23	35	43
	10%	—	—	—	—	11	22	29	41	10	22	33	41
	25%	8	15	21	30	7.5	16	24	30	11	22	31	38
	50%	6	11	16	24	4.5	10	16	23	4(?)	10	16	25
	75%	4	5	7	—	2	6	9	18	4	6.5	9	17
	100%	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium roqueforti	Air	28	62	91[2]	91[2]	23	61	85	90[2]	25	53	79	90[2]
	5%	—	—	—	—	18	52	89	90[2]	23	48	80	91[2]
	10%	—	—	—	—	14	49	81	91[2]	23	46	78	90[2]
	25%	17	44	52	93[2]	7.5	—	59	90[2]	20	48	77	92[2]
	50%	8	29	40	70	3	12	23	73	6.5	13	20	53
	75%	2	3	5	33	1	4	8	37	5	6.5	9	34
	100%	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium expansum	Air	18	37	—	—	16	33	50	68	12	27	43	60
	5%	—	—	—	—	15	33	51	67	16	29	49	66
	10%	—	—	—	—	15	31	46	63	15	32	47	63
	25%	8	17	25	38	8.5	19	27	43	14	27	40	58
	50%	5	9.5	13	24	3.5	7.5	12	29	4	8.5	13	30
	75%	2	4	5	16	3(?)	5	7	22	3.5	4.5	7	24
	100%	0	0	0	0	0	0	0	0	0	0	0	0
Endothia parasitica	Air	24	53	87	90[2]	4.5	34	65	88	12	38	68	90[2]
	5%	—	—	—	—	7.5	35	67	90	14	35	64	90[2]
	10%	—	—	—	—	7.5	33	60	83	11	34	62	90[2]
	25%	18	32	49	73	6	23	39	65	9	28	49	88
	50%	11	21	34	56	—	17	26	48	2.5	13	23	57
	75%	8	14	20	42	3	8	13	39	1.5	5	11	45
	100%	0	0	0	0	0	0	0	0	0	0	0	0

[1] The first three measurements in each case were made 3, 6 and 9 days respectively after exposure to the gas; the fourth was made 3 days after restoration of the cultures to ordinary air.

[2] Entire inside area of the plate covered with the colony, so that further growth was impossible. These values, therefore, are possibly too small, as the colonies may have reached the size mentioned before the close of the 12-day period.

Species	Gas Conc.	First Trial				Second Trial				Third Trial			
		3d Day	6th Day	9th Day	12th Day[1]	3d Day	6th Day	9th Day	12th Day[2]	3d Day	6th Day	9th Day	12th Day[2]
Endothia fluens	Air	21	55	92	—	8.5	43	73	86[2]	14	42	73	90[2]
	5%	—	—	—	—	11	42	71	87	8	28	54	83
	10%	—	—	—	—	7.5	29	51	73	11	32	56	87
	25%	9	12	34	60	5.5	22	39	67	7.5	22	38	71
	50%	7	9	21	45	1.5	10	17	37	0	9	25	42
	75%	1.5	6	11	30	0	2	6	27	0	2	7.5	34
	100%	0	0	0	0	0	0	0	0	0	0	0	0
Fusarium radicicola	Air	24	45	66	90	17	47	73	95[2]	22	44	70	90[2]
	5%	—	—	—	—	15	44	71	90[2]	20	43	66	91[2]
	10%	—	—	—	—	14	43	70	92	19	42	64	88
	25%	23	44	65	87	14	41	66	85	18	40	62	86
	50%	20	42	63	87	12	36	60	82	14	34	55	81
	75%	16	39	54	76	7	23	38	59	5	25	40	66
	100%	0	0	0	0	0	0	0	0	0	0	0	0
Glomerella cingulata	Air	29	54	80	91[2]	20	55	84	88[2]	22	50	75	90[2]
	5%	—	—	—	—	19	52	80	88[2]	22	50	74	90[2]
	10%	—	—	—	—	17	50	78	88[2]	24	47	70	90[2]
	25%	18	37	58	82	12	39	68	90[2]	24	44	63	85
	50%	15	29	44	67	9	26	43	69	10	23	35	57
	75%	10	19	30	53	5	16	29	55	6.5	13	23	52
	100%	0	0	0	0	0	0	0	0	0	0	0	0

radii not in a straight line was taken instead of the diameter). The medium used was potato glucose (2 percent) agar (3 percent). It was poured into the plates and the fungus inoculated into the center of the freshly hardened layer of agar. The plates were then sealed under bell jars and the gas introduced in the usual way. The apparatus was taken down every third day to record data. In the case of the test with pure gas the gas passed constantly through the jar containing the cultures. This dried the agar in the first trial, and it was thought that the dry condition of the agar might have had something to do with the failure of the fungi to grow after the restoration to the air. In the second and third trials, therefore, the gas was passed over water in a bottle before entering the jar; and this prevented evaporation of the moisture in the agar.

The accompanying table records the organisms, the treatments, and the results obtained, the measurements being diameters of colonies in millimeters. It can be seen from this that the lower limit of toxicity, or at least considerable retardation of growth, is between 10 percent and 25 percent in most of the species; but in one, *Fusarium radicicola*, it is difficult to locate, and is higher than for the rest of the

species. None of the values indicating retardation at 10 percent or below is significantly smaller than the value for its check except for *Endothia fluens* at about 10 percent. The lower limit for retardation of *E. fluens*, therefore, would seem to be somewhere between 5 percent and 10 percent. The retarded cultures, after being returned to the air, grew at approximately the same rate as the regular air cultures, thus demonstrating that after-effects are usually lacking; except that no development occurred in any case in any plate which had been exposed to pure gas.

Some of the cultures, including the two species of Endothia and *Fusarium radicicola*, produced in the toxic amounts of gas a more compact, velvety, deeper colony, with the hyphae more erect than in air. It is likely that these hyphae have interesting morphological characteristics induced by the treatment, but opportunity was not found to investigate this feature.

It seems clear, therefore, that among the organisms studied, including 13 species of bacteria and 11 species of fungi, there is no example of the extreme sensitiveness to illuminating gas which is displayed by some phanerogams.

2. Methane

Production and Purification of the Gas

It was found difficult to get methane of sufficient purity for the tests conducted. The best way to produce it is said to be to treat aluminium carbide with water; but owing to the war no aluminium carbide could be obtained. Some of the gas was prepared from methyl iodide by means of the copper-zinc couple. This gas was quite toxic to the organisms but had a distinct odor. It was therefore feared that some unchanged methyl iodide vapor passing over with the gas had been incompletely decomposed by the tower of zinc through which it had passed and that the reactions were due to this methyl iodide vapor. Some trials with methyl iodide vapor were accordingly conducted, and the results were corroborative of the fear just mentioned. The method finally settled on for preparing the gas with which most of the tests were conducted was the ordinary sodium acetate-soda lime method with barium oxide substituted for the soda lime. This method is said in some of the organic chemistry texts to produce nearly pure methane. In the earlier experiments

the gas was stored over water until required for use. Under these conditions the inhibitory effect of the gas was so slight as to create doubt as to its purity. Accordingly a sample was analyzed[3] and found to consist of a mixture of methane, hydrogen, oxygen, and nitrogen, of which the methane comprised about 50 percent and the nitrogen about 30 percent.

In order to get more exact data a mercury seal gasometer was secured for storage of the gas and each quantity of gas used was sampled for analysis. By this means dilution of the gas due to its solution in the water of the gasometer and the giving up of nitrogen to it by the water were avoided. It was also possible by means of the analysis to get an accurate measure of the concentrations used. The upper limit for the concentrations thus secured was 65 percent of methane with a maximum oxygen content of about 10 percent. A sample analysis follows:

CO_2	2.0%	CH_4	65.0%
C_nH_{2n}	0.5%	H_2	20.3%
O_2	10.3%	N_2 (difference)	1.9%

The results of the later tests confirmed the previously indicated low toxic properties of this gas as affecting the organisms in question. In fact, some of the previous tests gave rather stronger reactions on the part of the organisms than did the last ones in spite of the fact that all conditions indicate that the gas in the former trials was more dilute.

Effect on the Different Organisms

In these reports the results given are based on both the earlier and later work, care being taken not to make the gas seem less toxic than the data warrant.

Bacillus subtilis.—In all of the concentrations of methane used the development of *B. subtilis* was normal in character and good in amount. There was some checking of development in the higher concentrations which sometimes extended apparently as low as 25 percent (value uncorrected by analysis); but it was never great and was dissipated at the end of 3 days, except in a concentration of approximately 50 percent (corrected) or higher, where the slight retardation persisted in one or two cases for 6 days.

[3] The analyses of methane, carbon monoxide, and ethylene given in this paper were made by Prof. W. L. Badger and Mr. Philip W. Shepard, of the department of chemical engineering of the University of Michigan.

Bacillus pyocyaneus.—*B. pyocyaneus* grew well in all of the concentrations used, but with an almost indistinguishable retardation in percentages of 45 percent or above. This, however, had disappeared by the end of 3 days.

Bacillus Kieliensis.—The development of *B. Kieliensis* in methane was good throughout, but was slightly less vigorous in the greatest concentrations used. It was practically impossible, moreover, to determine the exact concentration at which the first inhibition could be said to occur. The color was also affected comparatively little, though it seemed in some cases to extend as low as 10 percent (corrected).

Bacterium stewarti and Sarcina lutea.—Contrary to expectations, these organisms were practically unchecked in all the percentages used except the very highest, about 60 percent (corrected); and even here the growth was good.

Oidium lactis.—The development of *O. lactis* was practically unchecked throughout the series of methane exposures. In the highest percentages, 50 percent to 65 percent (corrected), there was a tendency for the mycelium to have a white, velvety appearance instead of the more typically appressed, water-soaked appearance.

Cryptococcus Ludwigi.—The growth of this pink yeast was good in all concentrations of methane. It was usually slightly pale at 45 percent (corrected), somewhat pale and retarded at times at 50 percent (corrected), and distinctly pale and usually somewhat retarded at 65 percent (corrected).

Penicillium stoloniferum.—The growth of *P. stoloniferum* was slightly checked at concentrations of 45 percent to 65 percent (corrected).

As can be seen from the foregoing data, the toxic effects of methane on the organisms up to 65 percent of the gas are very mild and are certainly not of a character to suggest that 30 to 35 percent of methane in the illuminating gas used could be responsible for the inhibiting effect which illuminating gas exerts.

3. Ethylene

Production and Purification of the Gas

The ethylene used in these experiments was produced by heating 95 percent alcohol with c.p. sulphuric acid. It was passed through

wash bottles containing water, sodium hydroxide solution and c.p. sulphuric acid respectively. In the earlier work it was stored in a gasometer over water until required for use. The results were later checked by another experiment made with gas stored in a mercury seal gasometer. The following is the analysis of the gas used in this last experiment:

Acid gases, CO_2, SO_2, etc.	7.2%	
C_nH_{2n}	81.0%	
O_2	.3%	
CO_2	.3%	(or perhaps none)
Difference ($N_2 + H_2 + CH_4$)	11.2%	

Effect on the Different Organisms

The first nine of the following organisms were tested in 0.4 percent, 4–5 percent, 20 percent, 40 percent, 50 percent, 60 percent, 85 percent, and 100 percent (uncorrected values), except as otherwise stated, of ethylene. The concentrations, corrected according to analysis, which were used in verifying the results were 4 percent, 8 percent, 20 percent, 40 percent, 60 percent, and 80 percent. The percentages mentioned in connection with the first 9 species in the following notes are the corrected values unless otherwise stated.

Bacillus subtilis.—With *B. subtilis* there was no abnormality of colony type and no great checking of development in any concentration. There was none at all below 40 percent, and it was doubtful at that percentage. At 60 percent, however, it was unmistakable although not great.

Bacillus pyocyaneus.—Owing to the semi-translucent nature of the colony of *B. pyocyaneus* it was difficult to detect small differences in development, so that the lower limit for retardation as given may be somewhat too high. There was very little inhibiting effect exhibited, however; and the type of colony was not altered. The lowest concentration at which a retarding effect could be clearly distinguished was 60 percent, with a possibility that a very slight inhibition occurred sometimes at 40 percent.

Bacillus Kieliensis. There was little inhibiting effect exerted by ethylene on *B. Kieliensis* The organism grew vigorously in all the concentrations, and was quite often as vigorous as in air up to a concentration of 85 percent (uncorrected), but usually retardation could be detected at 40 percent and color variations at 20 percent or even sometimes at 8 percent.

Bacillus rubidus.—The concentrations in which *B. rubidus* was tested were 0.4 percent, 4–5 percent, 85 percent, and 100 percent, uncorrected values. There were no cultures with analyzed gas for verification. In the first three percentages the cultures were on autoclaved potato, in the last on agar. It grew in all, and when growing on the potato produced the characteristic orange-yellow pigment freely.

Sarcina lutea.—This organism was tested in 4 percent, 20 percent, 40 percent, 60 percent, 85 percent, and 100 percent ethylene, uncorrected values; and the results were checked with the percentages mentioned at the beginning of this section. The lowest concentration at which inhibition occurred was 40 percent, but good growth occurred in all. The color of the culture was never much affected.

Oidium lactis.—This organism grew well in all the gas concentrations used, the cultures being scarcely distinguishable up to 60 percent from those in air. At greater concentrations slight inhibitive effects were noted.

Cryptococcus Ludwigi.—This yeast grew well in all of the tests although the development was checked and the color paler in the higher concentrations. The lowest percentage at which retardation was unmistakable was 40 percent. At 80 percent the growth was quite slow at first and the color nearly lacking. In a few days, however, the development became greater and the color darker, although not equaling that in the air until the return of the culture to the air.

Penicillium stoloniferum.—This species was tested in 4 percent, 85 percent, and 100 percent, uncorrected values, of ethylene, and the results were verified with analyzed gas as indicated above. The organism grew well in all concentrations but was slightly checked at 40 percent and increasingly so as the concentration increased.

Bacterium stewarti.—This species was tested in 50 percent and 85 percent, uncorrected values, of ethylene and the results verified with analyzed gas as reported above. It grew well in all concentrations used. In the early stages of the exposures there was inhibition at as low a percentage as 60 percent or perhaps at 40 percent but, by the third or fourth day the effect had disappeared in all.

The following species were tested, two trials each, in only 50 percent and 85 percent, uncorrected values, of ethylene. There was no confirmatory test with the analyzed gas.

Bacillus carotovorus.—The development of *B. carotovorus* while

exposed to ethylene was good in all cases tested. In 50 percent of the gas it was quite equal to the air culture and in 85 percent it was only slightly less vigorous.

Bacillus melonis.—In one trial the growth of *B. melonis* in both ethylene contents was about equal to that in air. In the other there was slight inhibition in the 85 percent concentration.

Bacillus campestris.—The tests seemed to show a slight inhibition of *B. campestris* at 50 percent of ethylene although they did not agree especially well on the point. At 85 percent the inhibition was somewhat greater but not at all remarkable.

Bacterium tumefaciens.—With *B. tumefaciens* the tests showed a clear inhibitive effect at both concentrations of the gas but greater at 85 percent than at 50 percent, and the effect was maintained until the cultures were removed to the air.

Bacillus solanisaprus.—The growth of *B. solanisaprus* in the two ethylene-air mixtures was practically equal to that of the same organism in air.

Bacillus radicicola.—The development of the check culture of *B. radicicola* in air and of the cultures in 50 percent and 85 percent ethylene were practically identical in both tests.

Bacillus mycoides.—In the first test with *B. mycoides*, where the inoculation was made in a streak, no difference could be made out between the growth in air and in the ethylene. In the second test, however, where the slant was inoculated at a single spot near the center, it took about four days for the colony to spread over the entire slant in 85 percent ethylene, and about three days in 50 percent ethylene, while in air the invasion was complete in two days.

It seems quite clear to the writer from the results mentioned above that the presence of 4–5 percent of ethylene in illuminating gas is totally inadequate to account for its effect on the bacteria and fungi studied.

4. Carbon Monoxide

Production and Purification of the Gas

The carbon monoxide used in these studies was made by heating crystallized potassium ferrocyanide with c.p. sulphuric acid and a little water. It was bubbled through sodium hydroxide solution to remove the small amounts of CO_2 and SO_2. In the earlier work it

was stored over water until desired for use. At the last, one experiment was run with gas which had been stored in a mercury seal gasometer and so had not been subjected to the alteration of composition due to absorption by the water and the giving up to it of gases in the water. The results of the last test were corroborative of the former ones. The analysis of the gas used in this last test is here given.

CO_2..... 1.0%	O_2.... 1.2%	N_2 (difference).... 5.7%	
C_nH_{2n}.... 0.4%	CO... 91.7%		

In view of the high percentage of CO in the gas used it has not seemed desirable to correct the values by calculation. The gas used in the earlier tests was probably lower in CO than that used in the last one. For this reason the results obtained in the last test have been used instead of the others where a variation occurred which seemed to be due to a higher CO content in the last quantity of gas.

Effect on the Different Organisms

Bacillus subtilis.—No effect of carbon monoxide on *B. subtilis* could be observed at 10 percent or below, but at 25 percent and above the same sort of very thin colony was produced as was produced in illuminating gas at the higher concentrations. As in the case of illuminating gas, also, the colony did not usually undergo change to the normal air type when returned to the air, but often a normal colony would start up at some point and invade the slant from that center. Using this organism as a measure of toxicity, therefore, carbon monoxide would appear to be something like twice as toxic as illuminating gas.

Bacillus pyocyaneus.—This organism grew in all the concentrations of carbon monoxide used although its development was slowed down to some extent in the higher percentages. It was difficult to determine any definite place at which the inhibition set in, owing to the indefinite tint of the colonies, although it seems likely that it should be placed at about 25 percent.

Bacillus Kieliensis.—*B. Kieliensis* was found capable of growing in all the test conditions with carbon monoxide. The first pronounced retarding effect occurred at 25 percent, although it was small and not altogether uniform. The color was sometimes paler at 10 percent and usually so at 25 percent, while at 75 percent and higher the

pigment was usually nearly or quite lacking and the development very slight. It will be noted that if we take *B. Kieliensis* as a test organism carbon monoxide appears to be just about as toxic as illuminating gas.

Bacillus rubidus.—In the case of *B. rubidus* a single successful series of cultures with carbon monoxide showed a retardation in the rate of development at 10 percent; at 25 percent it was quite marked; at 50 percent and 75 percent the development was very slight at the end of a 5-day period; and at 100 percent (uncorrected) there was no visible development. After return to the air vigorous growth took place in 2 to 3 days. This organism was not included in the supplementary test with the gas that was stored in the mercury seal gasometer.

Sarcina lutea.—The development of *S. lutea* was normal or nearly so to 10 percent of the gas but at 25 percent the inhibitive effect was clearly noticeable. At 50 percent to 75 percent the development was very slight during an 8-day period of exposure. At 100 percent (uncorrected) there was very little if any discernible growth.

Oidium lactis.—Carbon monoxide exerted a definite checking effect on *O. lactis* at a concentration of 25 percent and a possible very slight effect at 10 percent. The development was good at nearly 100 percent, however, and only slightly atypical in character. There was, however, a slight tendency in the gas for the hyphae to grow upward and assume something of a tufted character.

Cryptococcus Ludwigi.—This organism grew in all the percentages of the gas used, although slight inhibition occurred at 10 percent and the development was slight or very slight in the undiluted gas. With the inhibition of growth went also decrease in depth of color, so that in the case of the greatest inhibition the colony was practically colorless. The colonies in the gas up to 75 percent reached a maximum quite as great as that in the air but took a few days longer, while for colonies in an atmosphere containing more carbon monoxide neither this maximum nor the typical intensity of color was reached. Upon return to the air, however, these conditions were attained.

Penicillium stoloniferum.—The lowest CO content at which growth was checked was 10 percent. At this percentage the checking was very slight, but increased with increase in the CO content, so that growth was very slow at 75 percent and above although conidia were usually produced, and if not they followed promptly on return of the culture to the air.

From these results it would appear that carbon monoxide is approximately equal to illuminating gas in the inhibitive effects on the organisms tested and considerably more active in this way than either methane or ethylene. It would not seem to be sufficiently toxic, however, to be the sole cause of the effects produced by the illuminating gas.

5. General Observations and Discussion

It was noted in the present work, as was of course to be expected, that not all organisms showed the same sort of reaction or exhibited the same degree of tolerance to the gases. Thus *B. subtilis*, *B. pyocyaneus*, *B. mycoides*, and *B. Kieliensis* showed a high degree of tolerance for illuminating gas. In the case of *B. subtilis*, *B. mycoides*, and *B. Kieliensis* the colony in the high concentrations was quite different in appearance from the normal one. In the case of the first two species this is owing perhaps merely to the very small mass of material produced, but in the last named it is associated also with a decrease in pigment production. However, in the case of *B. pyocyaneus* and *O. lactis* the appearance of the colony was comparatively little altered. Probably the most sensitive of the species studied were *Sarcina lutea*, *Bacterium stewarti*, and *Penicillium stoloniferum*. Among the group of fungi tested together, *Fusarium radicicola* was the most resistant while *Endothia fluens* was most inhibited by the lower percentages of gas, followed by *E. parasitica* and *Penicillium pinophilum*.

The data do not seem to warrant any conclusion that any of the strains acquired an increased degree of tolerance for illuminating gas by being cultivated continuously in its presence. Such did seem to be the case for a time with *Bacillus pyocyaneus* and *Bacterium stewarti*, but when cultures inoculated with the original mother strain, which had not been exposed to gas at all, were exposed to the gas along with the supposed acclimated strain, the development of the unacclimated strain was quite equal to the other. In fact there was some evidence that continuous growth in toxic concentrations of the gas weakened the organism slightly. This was more clearly evidenced with *Bacillus Kieliensis*, perhaps, than with any other and was shown by the fact that the color production was not quite normal for three or four transfers in the air after several transfers in pure gas.

It is hardly possible at this time to state definitely just what causes the inhibiting action of illuminating gas. Some checks run with hydrogen, carbon dioxide, and air washed in pyrogallol indicated rather strongly that a good part of it is due to the lack of oxygen, even with the facultative anaerobic species. Not all of the results can be so accounted for, however, as it does not explain the after-effects, nor why one gas in a given concentration should produce a greater effect than another, as, for instance, why a mixture of 25 percent carbon monoxide and 75 percent air should produce almost as great a retarding effect on the growth of *B. subtilis* as a mixture of 50 percent illuminating gas and 50 percent air. Certainly no one component of the illuminating gas has toxic properties sufficient to account for the results. Ethylene and methane are relatively innocuous, and in addition ethylene is present in only small quantities in illuminating gas. There is also to be considered the possibility that some of these compounds, especially methane, may serve as food material for the organisms. In this connection the work of Münz (12) is suggestive since it shows that some bacteria are capable of assimilating methane. Carbon monoxide proved to be more toxic than the illuminating gas in some cases, but it also is present in only small quantities. What appears to be the most reasonable hypothesis for the present is that the results are the sum of a relatively large effect due to the dilution of the oxygen plus a smaller effect due to the weakly poisonous properties of some of the component gases, the most important apparently being carbon monoxide.

In view of the very great toxicity which illuminating gas and ethylene show toward many phanerogams, it was a distinct surprise to the writer to find his cultures showing uniformly such a high degree of tolerance to these gases. The comparative degrees of tolerance can perhaps be better realized by a brief consideration of the general results for the two kinds of plants. One of the recent studies on the effect of illuminating gas and its constituents on some phanerogams and higher green cryptogams (*Doubt*, 3) included a large number of species in the plants tested. The concentration of gas at which reactions first occurred in some of the most sensitive species was 25 parts per million (0.0025 percent), while the only species not affected at 60,000 parts per million (6 percent) were species of Polypodium, Aspidium, and Asplenium, although some others which were affected could live at that concentration. In all of the phanerogams tested

at this degree of concentration reaction was obtained. In the case of the 25 cryptogams reported in the present paper, however, there was uniformly no visible reaction at 5 percent. So far as present records go, therefore, the phanerogams which are most tolerant to illuminating gas are not more tolerant, indeed are apparently less so, than the most sensitive bacteria and fungi. In other words, the least sensitive phanerogams are more sensitive than the most sensitive bacteria and fungi. It was also a matter of surprise, in view of the extreme toxicity of ethylene to phanerogams, to find that it is relatively innocuous to the cryptogamic species studied. In this case carbon monoxide was found to be considerably more toxic than any other organic constituent of illuminating gas. The fact that ethylene is more toxic to one group of plants and carbon monoxide to the other is further evidence of a great difference in the sensitivity of the two groups.

Incidentally it may be remarked that so far as the results from the species studied in this investigation can be projected to cover all species, they indicate that there is only a small chance that the gas which would escape from the gas fixtures in a room would be enough to invalidate results obtained from cultures in that room. It should be remembered, however, that even phanerogams vary considerably in this regard, as has recently been shown by Miss Doubt (3) and others; also that at least some algae appear to be quite sensitive, as is reported by Woycicki (17, 18). It would not be at all surprising, therefore, and is perhaps to be expected, even, that some more sensitive bacteria and fungi will yet be found. The known existence of only a few such would render precautions necessary in bacteriological and mycological work which in the light of the results here reported seem unnecessary.

CONCLUSIONS

1. None of the species of cryptogams studied, including 13 bacteria and 12 fungi, shows any very marked sensitiveness to small amounts of illuminating gas or its components.

2. In the higher concentrations (25 percent and above) of the gas and its components, however, most of the bacteria and fungi used are checked in growth or wholly stopped. In the latter case growth will usually take place after exposure to the air, although often from a comparatively few foci, as if many of the cells had been killed. Sometimes the culture is entirely sterilized.

3. Different species exhibit different degrees of tolerance for the gases, and in general a species which is relatively intolerant of one is relatively intolerant of others.

4. There was no real evidence that the continued culture of an organism in illuminating gas induces the development of an increased tolerance for the gas by the strain so cultivated. On the other hand there was some slight indication that the vigor of a strain so cultivated is slowly lowered.

5. The colony habit of organisms is often modified more or less strikingly in the more toxic gases. This is exemplified especially in the color variations of *B. Kieliensis*, the decrease in colony mass and gross appearance in *B. Kieliensis*, *B. subtilis*, and *B. mycoides*, and the more compact, upright arrangement of hyphae in several fungi.

6. Ethylene and methane are relatively less inhibitory to the organisms used than is illuminating gas, but carbon monoxide is about equal to the illuminating gas in this respect.

7. The effect of the gas cannot be laid to any one constituent, but is probably the sum of the small effect of each plus the greater effect of a deficient oxygen content.

8. Incidentally the foregoing results indicate that the amount of illuminating gas often present in laboratory air is not a menace to scientific results in bacteriology and mycology.

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UNIVERSITY OF MICHIGAN,
ANN ARBOR, MICHIGAN

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